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HEALTH INTELLIGENCE FOR FUEL AND FUEL ADDITIVE REGISTRATIONS

National Environmental Research Center
Research Triangle Park, North Carolina

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Health Intelligence
for
Fuel and Fuel Additive Registrations

An EPA Task Force Report

Division of Health Effects Research
National Environmental Research Center
Research Triangle Park, N.C. 27711

March 15, 1972

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I. INTRODUCTION

This task force report resulted from meetings initiated by the Division of Health Effects Research and held at Research Triangle Park on February 29, March 1 & 2, 1972. The purpose of the task force was to develop a systematic plan to provide health intelligence required under Section 211 ("Regulation of Fuels") of the Clean Air Act of 1970, as amended. Section 211 gives EPA authority to require the conduct of tests "to determine potential public health effects" of fuels or fuel additives designated for registration and to control or prohibit use of fuels or additives when emission products "will endanger the public health or welfare". Through this report, the task force has suggested a program to carry out the above responsibilities.

The members of the task force were as follows:

Office of Fuel and Fuel Additive Registrations	Henry Miller
Division of Chemistry and Physics	John Moran
Division of Health Effects Research	John Finklea (Chairman) Ferris Benson Kenneth Bridbord Kirby Campbell David Coffin Anthony Colucci Marvin Hertz Wellington Moore Carl Shy Wayne Sovocool

II. RECOMMENDATIONS

1. The Task Force concerned with providing health intelligence for the Office of Fuels and Fuel Additive Registrations (OFFAR) recommends that a multidisciplinary committee be formed, composed of personnel from the Office of Fuel and Fuel Registration, a combustion chemist, an analytical chemist, a biochemist, a toxicologist, an ecologist, a physician and others as deemed appropriate. The committee will be concerned with the systematic assessment of possible hazards to public health and welfare from fuels and fuel additives submitted for registration.

2. Industry should be responsible for conducting all tests required to characterize exhaust emissions resulting from use of registered fuels or additives.

3. Standard protocols to assess biological effects of exhaust emissions resulting from use of registered fuels and fuel additives should be developed and tested during FY 73.

4. An additive causing significant changes in vehicular emission patterns or having toxic potential in its emission products should be subjected to an initial toxicologic (including phytotoxic) screen. Development of the toxicologic screen should receive high priority early in FY 73.

5. A full toxicological evaluation should be selectively required for those additives whose initial screening suggests further investigation.

6. Results of toxicological screening and more definitive animal toxicological studies should be selectively evaluated through clinical and epidemiological human studies.

7. New legislative authority should be sought authorizing OFFAR to obtain quantitative fuel and fuel additive production information and usage data from manufacturers.

8. Disclosure of information acquired by industry from toxicologic testing or occupational exposures during the course of manufacture and distribution of fuels and fuel additives should be required. This disclosure could be required through a change in existing regulations authorized under Section 211 of the Clean Air Act.

9. A tax of 1/100 cents per gallon on retail sales of fuels is recommended to cover the cost of all toxicological testing. This tax would generate $13,000,000 per year and would provide support to the entire fuel and fuel additive registrations program. All toxicological testing should be carried out under EPA, rather than industry, supervision.

III. NATIONAL SIGNIFICANCE

The 107 million motor vehicles presently registered in the United States will consume about 130 billion gallons of fuel per year. Gross retail sales of this volume of motor vehicle fuel amounts to $48 billion annually. Currently, 300 gasoline fuel additives have been registered with the Office of Fuel and Fuel Additive Registration. In 1969, 870 million pounds of additives were consumed at a gross value of $280 million. Under proposed new regulations requiring registration of gasoline, diesel fuel, fuel and heating oils, aviation fuels, crank case lubricants, liquified petroleum gas and additives, a total of 1000 to 1500 additives are likely to be registered. Seventy-seven million tons of motor vehicle pollutants are emitted annually in the United States. This pollutant mix can be generally characterized as follows:

Chemical Class	Emission Rate (tons/year)
Combustible particulates*	380,000
Carbon monoxide	55,000,000
Hydrocarbons	13,600,000
Polynuclear aromatics	480
Nitrogen oxides	7,600,000
Lead	230,000
Sulfur oxides	280,000
Total	77,090,480

*Includes trace elements

The Environmental Protection Agency has received a mandate from Congress, expressed in Section 211 of the Clean Air Act of 1970 as amended, to control or prohibit the manufacture or sale of any fuel or fuel additive when emission products will endanger the public health or welfare or will impair the performance of any emission control device (Section 211, c.1.). Under this Section of the Act, EPA may by regulation require registration of any fuel or fuel additive

prior to sale or introduction into commerce. Registration requires information on the chemical composition and range of concentration of any additive in fuel. EPA may also require from manufacturers information "*as is reasonable and necessary* to determine the emissions resulting from use of fuels and additives" and "the extent to which such emissions affect the public health or welfare" (Section 211, b.2.B.).

The authority and mandate delegated under Section 211 *require EPA to evaluate the potential public health effects* of registered fuels and fuel additives. The following problems were identified and considered by the Task Force in planning to meet this challenge:

1. At present, no EPA person or committee systematically scrutinizes each registered fuel additive for potential public health effects.
2. EPA lacks a systematic program to evaluate the toxicologic potential of registered fuels and additives.
3. Prime concern *should not* be focused on toxicity of parent compounds registered by the Office of Fuel and Fuel Additive Registration. The chemical composition of these compounds will undergo drastic changes in the course of combustion and passage through advanced emission control systems. The public will be primarily exposed to these combustion products rather than to parent compounds. At present, the manufacturer supplies no information on exhaust products.
4. However, occupational exposures to parent compounds do occur as a result of evaporative losses during manufacture and handling of fuels and additives. In many cases, manufacturers have large, excellent occupational health and safety programs, through which

human exposure data are acquired. This information is often proprietary and is not made available to the Government. Protection of the health of fuel and additive handlers, including service station operators and to a lesser extent the general public, could be considerably furthered by requiring transmission of existing occupational health data to the Government. This effort could be coordinated through the National Institute of Occupational Safety and Health, DHEW.

5. The following compounds have been identified in motor vehicle exhausts:

TABLE. Compounds Identified in Motor Vehicle Exhausts

I. Particulate Emissions

A. Lead and Organics

$Pb(Cl, Br)_2$
$Pb(NH_3)_2$ & $Pb\,NH_3\,Cl$
$PbO \cdot Pb(Cl, Br)_2$
$PbO \cdot PbSO_4$
$PbCl_2$
$PbBr_2$
Organics (PNA's, etc.)
HCl
Fe_2O_3
Fe_3O_4
NH_4Cl

B. Other Trace Elements Emitted in Exhaust Particulates*

Fe	Mg
Ni	Mn
Cu	Cr
Al	Sn
Ca	Zn
Si	Ti
Sb	Mo

II. Gaseous Emissions (based on 23 minute test cycle)

PNA (polynuclear aromatics)
NO
NO_2
CO
CO_2
200 Hydrocarbons
Aldehydes and Oxygenates

*List is not exhaustive; other trace elements are being identified.

Emission controls on 1975 and 1976 vehicles should reduce hydrocarbon and polynuclear aromatic emissions by a factor of 100 from 1966 levels. These hydrocarbons are likely to be broken down almost completely to CO_2 and H_2O. Advanced catalytic control systems employing reducing catalytic reactors are likely to achieve substantial reduction of nitrogenous compounds but may result in emissions of ammonia and organic nitrogen compounds. In addition, trace metals in fuels and additives and in deterioration products of catalytic controls will be emitted. The Task Force agreed that the following pollutants, likely to occur in emission of fuels combusted through catalytic reactors, have significant toxic potential:

a. Trace metals
b. Ammonia and other nitrogenous compounds
c. Organic particulates and gases (at 1/100 of 1966 levels)

Of most immediate concern are trace metals in blended or raw motor vehicle fuels and known to be present in exhaust particulates. The Task Force agreed that these trace metals could be ranked from highest to lowest in terms of potential public health hazard and exposure levels as follows:

Element	Relative Concentration (% of particulate fraction)
1. Manganese	0.10
2. Nickel	0.02
3. Cobalt	0.02
4. Chromium	0.05
5. Tin	0.02
6. Antimony	trace
7. Molybdenum	0.01
8. Titanium	0.02

These elements possess the potential for accumulating in human tissues and producing definite systemic toxicity at elevated tissue levels. Other trace elements of low toxic potential in fuels and exhaust particulates include iron, calcium, zinc, silicon, copper and aluminum. Cadmium is reported at trace amounts in some fuels, but this finding is disputed. Barium, lithium and boron are fuel additives which are not known to be in raw fuels. Of these three elements, barium has the highest toxic potential. In general, systematic studies of these trace elements have not been conducted, and EPA is not in a position to take regulatory action based on health intelligence.

6. The Task Force recognized the need to acquire emission profiles, chemically characterized, of particulates and gases resulting from combustion of fuels and their passage through advanced emission control systems. Profiles must be acquired for reference gasolines to serve as a comparison for changes caused by use of registered fuel additives. The Task Force further recognized that these emission profiles would demonstrate a complex array of chemical constituents. The array could change markedly with irradiation or with blending of each new fuel additive. Evaluation of the toxicity of individual constituents would be a costly and tedious task, whereas the toxicity of the complex whole is the single important consideration. Therefore, as a first step, a system is needed to evaluate comparative toxicologies of reference fuels against fuels with specific additives at concentrations approximating anticipated ambient exposures.

7. An inexpensive rapid toxicologic screening system is needed to obtain information on potential toxicity of exhaust particulate profiles for which little or no biological effects data exists. This screening system must employ standardized protocols using proven toxicologic methods. Methods to provide exhaust particulates for these tests are also required.

8. Depending on the results of toxicologic screening, more definite toxicological studies of exhaust particulates resulting from use of fuel additives should be selectively applied. Protocols for these evaluations are required and must be standardized as soon as possible in fiscal year 1973.

9. Trace elements of significant toxic potential in fuels and additives should be evaluated in elemental or simple form by inhalation exposures of whole animals. These substances will pass through advanced emission control systems; hence, their presence in fuel additives requires assessment both in isolated form and within the particulate exhaust complex.

10. The Task Force recognized that fuel additive composition may affect emission rates of carbon monoxide, nitrogen oxides and precursors of photochemical oxidants. The health effects of these compounds are presently under intensive study within the Division of Health Effects Research.

IV. MEETING THE CHALLENGE

Registration and testing procedures must minimize the risk of adverse human health effects that can reasonably be attributed to differences in fuels or fuel additives. Such effects may be linked to individual additive components, complete additives, blended fuels or gaseous and particulate emissions from mobile and stationary sources. Effects of concern include, but are not limited to an increased risk of cancer and mutations, impaired reproductive performance, increased susceptibility to respiratory infection, increased human pollutant burdens and subtle changes in cellular morphology and enzymatic function. A schematic overview of the public health impact of fuels and fuel additives may be visualized in Figure 1. The effect of individual additive components may be evaluated by reviewing industrial toxicologic screening data compiled during research and development for new products. Routine occupational health studies and specialized surveillance of industrial exposures can be utilized to evaluate the hazards associated with the direct exposure to complete additives and to blended fuels. Such exposures occur during additive and fuel manufacturing, during distribution and, to a lesser extent, during consumption. However, the primary concern of the Task Force was the impact of differences in fuel and fuel additives upon gaseous and particulate emissions which can affect the general population, segments of which may be especially susceptible. As previously discussed, necessary research on the direct effects of carbon monoxide, oxides of nitrogen and particulates per se have been considered by the Health Planning Task Force and in other program elements. On the other hand, our Agency has devoted limited resources to research on the health effects of gaseous and particulate organic compounds, trace elements, ammonia, and other nitrogenous compounds. These are the pollutants that may be qualitatively and quantitatively altered by differences in fuels or fuel additives.

Figure 1

Overview of Public Health Impact of Fuels and Fuel Additives

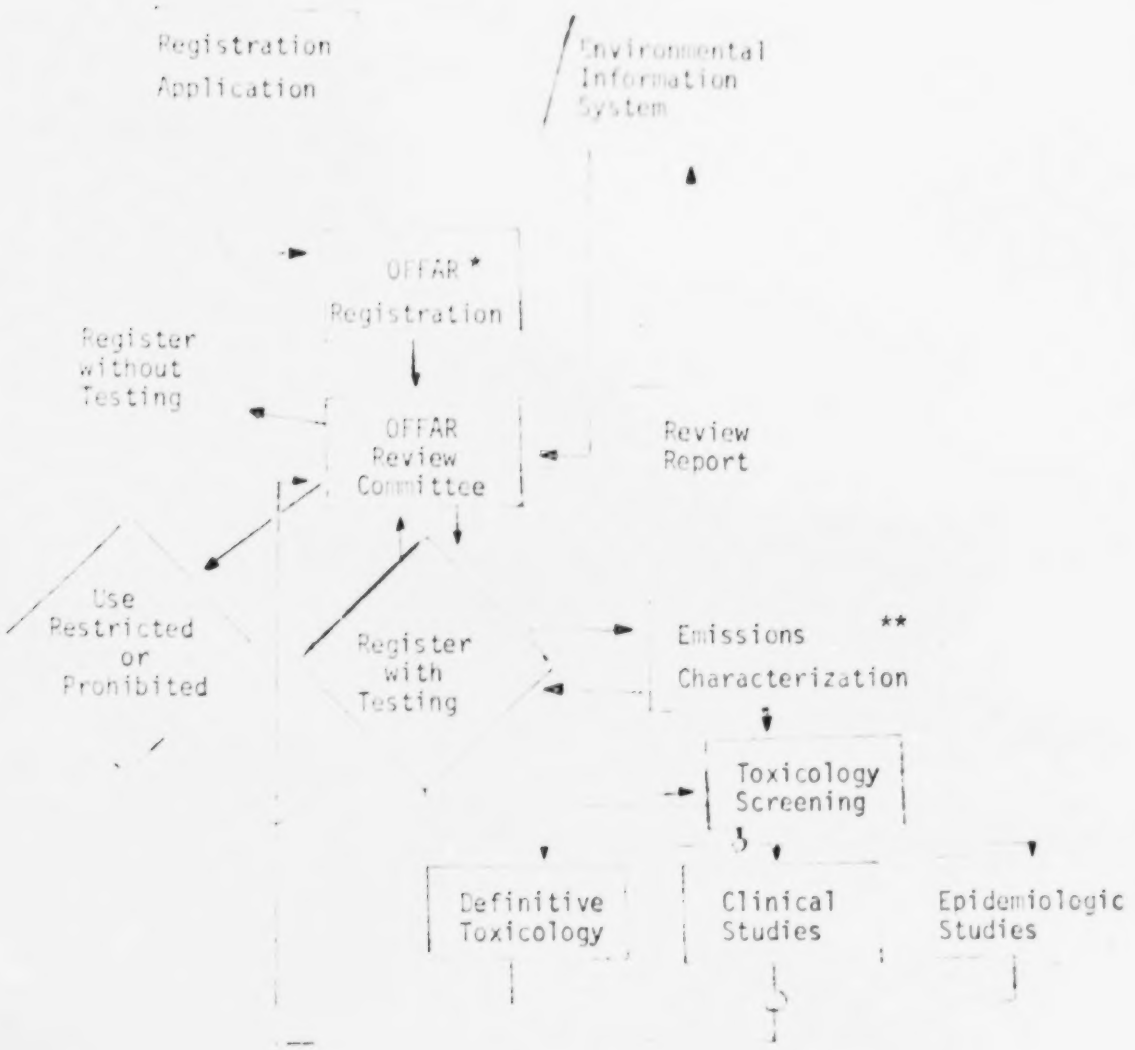
Factor Considered	Production and Consumption Stages			
Fuel or Fuel Additive Component	Research and Development →	Manufacturing →	Distribution and Vending →	Consumption
Emissions of Concern	Additive Components	Additive or Blended Fuel	Evaporative Losses of Blended Fuel	Gaseous and Particulate Emissions from Mobile and Stationary Sources
Human Exposures	Workers	Workers	Workers	General Population
Health Intelligence Research Approaches	Industrial Toxicology Occupational Health	Occupational Health	Occupational Health Special Toxicology	Toxicology Epidemiology

A systematic effort is required to evaluate the public health risks which may result from emission changes and to assure human safety through the registration mechanism. Such a system is schematically presented in Figure 2. Registration applications containing relevant industrial toxicology and occupational health summaries would be received by the Office of Fuels and Fuel Additive Registration (OFFAR). These would then be screened by a Review Committee composed of a representative from OFFAR, an ecologist, a toxicologist, a biochemist, a representative of the Office of Research and Monitoring, a representative of the Office of Air Programs, and others as deemed appropriate. The OFFAR Review Committee would consider each fuel and fuel additive registration and registration application. Some fuel additives or specific fuels would be selected for testing on the basis of widespread usage, predicted survival of a potentially toxic substance, predicted effects upon gaseous and particulate emissions, impact on pollution control devices, alteration of atmospheric reactivity and visibility, and postulated public health and welfare hazards including damage to plants and wildlife.

In general, the Committee would first require characterization of emissions according to standard protocols now being developed by the Division of Chemistry and Physics of the Research Triangle Park National Environmental Research Center. The results of this testing would be evaluated by the Review Committee and toxicologic screening would be requested when indicated by significant qualitative or quantitative changes in emissions of gaseous hydrocarbons, trace elements, organic particulates, ammonia or other nitrogenous compounds. Should toxicologic screening tests be positive, more definitive investigations could be instituted including further toxicologic testing, special studies of occupational groups, appropriate exposure of human volunteers and epidemiologic studies of populations representing an exposure gradient for the pollutants in question. The results of these tests

Figure 2

Assessment of Public Health Impact
of Fuels and Fuel Additives



*Office of Fuel and Fuel Additive Registrations

**Phytotoxic screening could most easily be performed concurrent with emissions characterization

would be reported to the Review Committee which would make recommendations on the use of a particular fuel additive or fuel. On occasion the Review Committee might simultaneously initiate tests involving emissions characterization, toxicologic screening and even more definitive toxicologic and human studies. The Review Committee would utilize inputs from an environmental information system and submit reports to such a system.

A more detailed overview of the proposed toxicologic screening system, shown in Figure 3, reveals four levels of complexity. A rough index of toxicity will first be established by comparing the response of tissue culture cell lines exposed to suspended particulate matter collected from reference fuels and similar fractions collected during emissions characterization testing. Both morphologic and biochemical endpoints would be utilized. When such screens indicate significant increase in toxicity, or when the Review Committee deems appropriate, more specific screening at two levels of complexity may be initiated with endpoints involving susceptibility to infection, carcinogenesis, mutagenesis, and reproductive efficiency. In vitro enzymology profiles would then be obtained on those materials which had caused significant adverse effects. Enzymology profiles will help elucidate mechanisms of action and will provide crosswalks to relate more definitive toxicologic testing and human studies to the toxicologic screening tests.

Toxicologic testing of appropriate compounds of trace elements would involve the steps shown in Figure 4. Trace element aerosols of interest would be generated by the same propane-combustion system that is currently utilized in human exposure studies of lead. One or more species of animals would be exposed. Routine tests would involve metabolism and tissue burdens, biochemical and pathological changes, and assessment of physiologic function. Special toxicologic testing would also be instituted where appropriate. Definitive toxicologic testing of the carcinogenic,

Figure 3.

Toxicologic Screening of Fuels and Fuel Additives

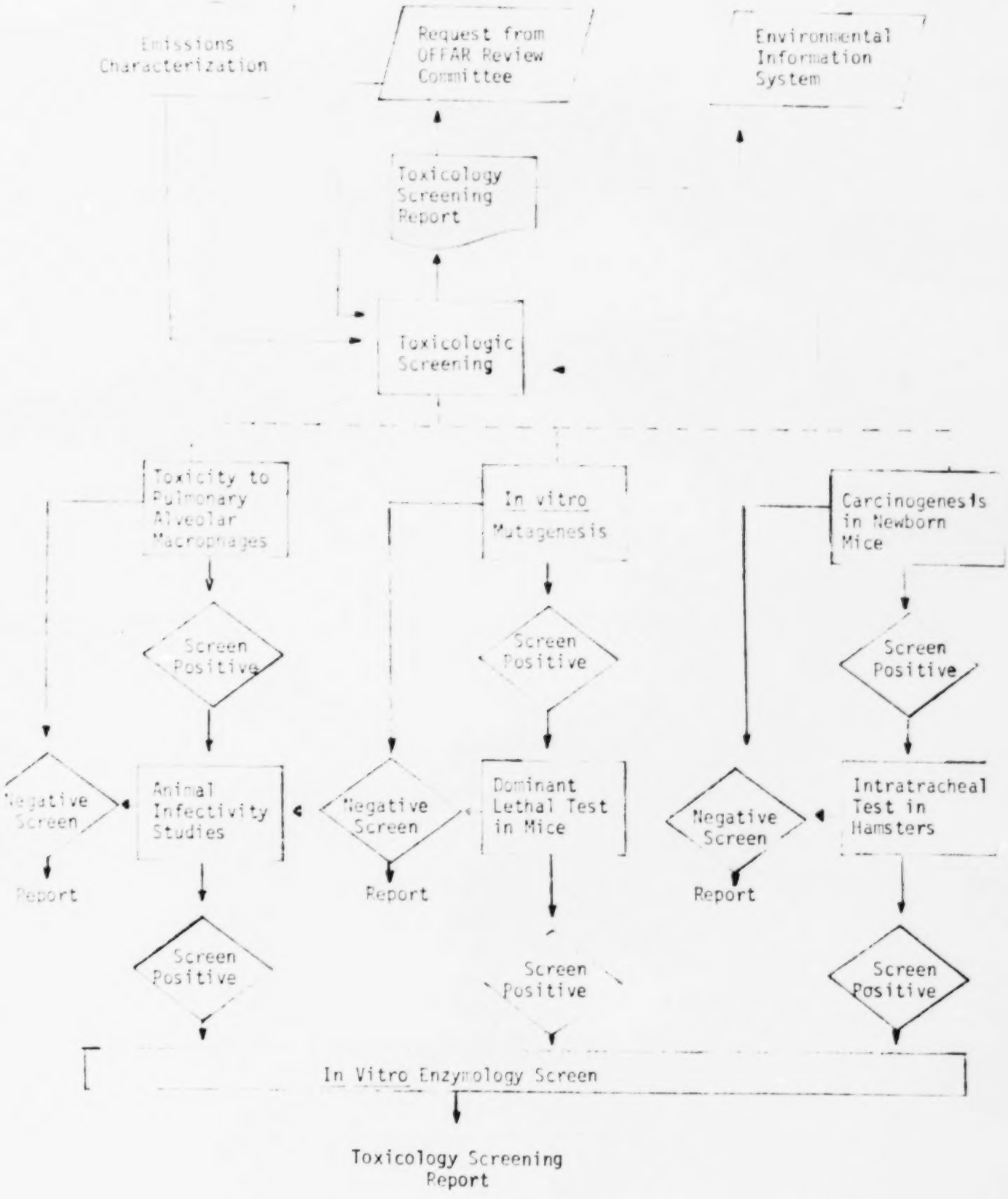
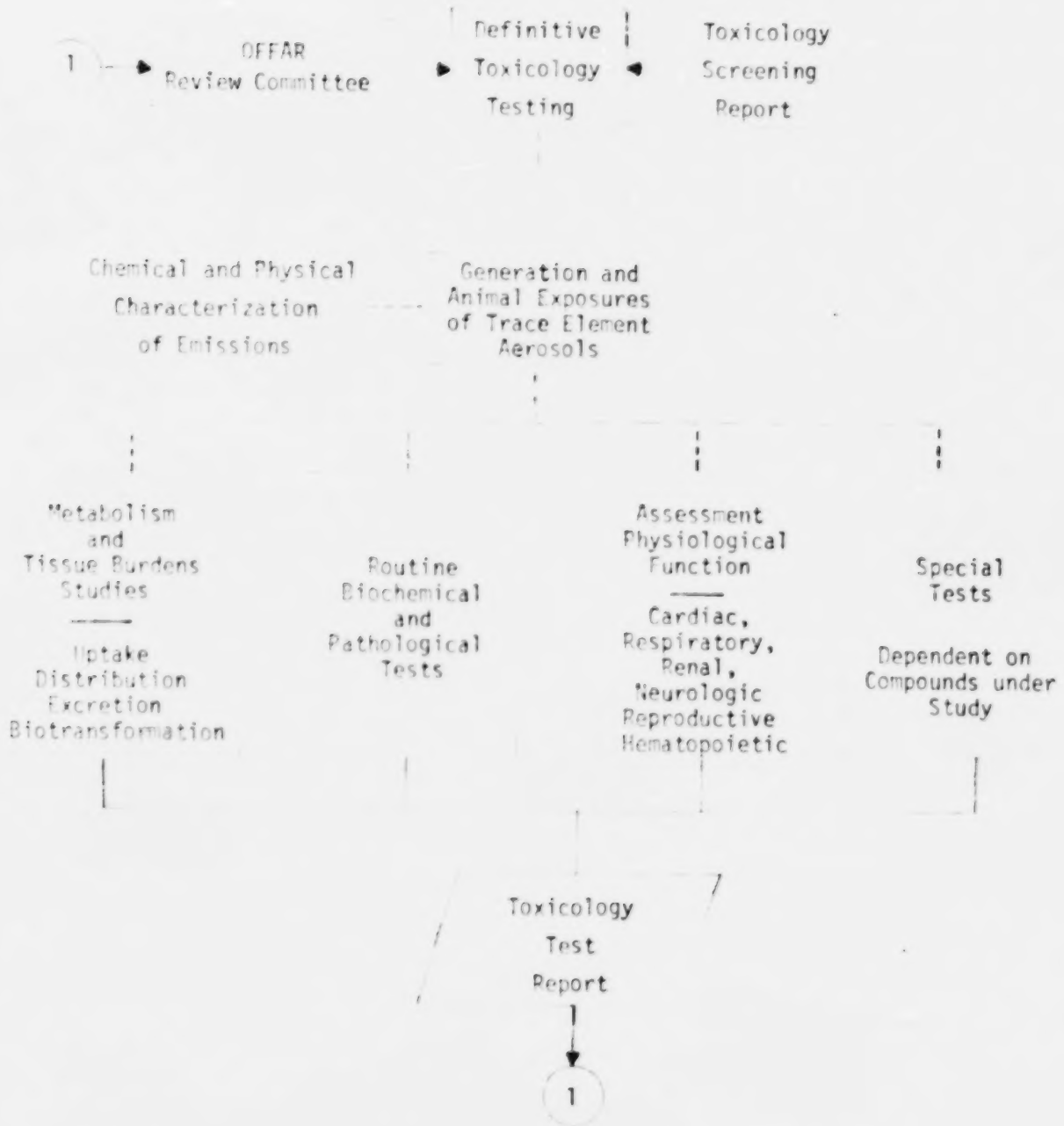


FIGURE 4. Definitive Toxicologic Testing of Trace Elements Found in Fuels and Fuel Additives



mutagenic and teratogenic hazards associated with specific organic fractions or compounds could be requested from the National Center for Toxicologic Research or performed by other EPA toxicology groups utilizing protocols recommended by the National Academy of Sciences.

Epidemiologic studies, investigations involving accidental or occupational exposures and controlled exposures of human volunteers would be most appropriate when evaluating the potential hazards attributable to toxic trace metals and hazards associated with evaporative losses or occupational exposures.

Task statements found in the Appendix detail the necessary steps and resources for the described OFFAR activities to meet the scientific challenge of fuel and fuel additives.

V. BUDGET IMPACT

At present only $225,000 is budgeted from the Fuel and Fuel Additive Registration program element for health effects research. Two other component tasks of the proposed system, Effect on Emission Products (Task 2) and Determination of Fuel and Fuel Additive Usage Patterns, (Task 3), are scheduled to receive $885,000 from the same program element. Another system component, Toxicologic Testing of Trace Elements (Task 7) will receive limited support from the Biomedical Research program element.

The present budget will allow design and testing of one fuel additive in part of the proposed toxicologic screen. The present budget will not support establishment of a biochemical effects screen or any human studies. A minimally adequate budget for health effects studies of fuels and fuel additives would total $1,115,000 and an optimal budget would amount to $2,925,000. At the minimally adequate level, the toxicologic and biochemical screening systems would be established in FY 73 and 10 fuel additives would be screened. At the optimal level, 20 fuel additives would be screened and needed human studies of trace elements completed.

The man-years of effort and dollar resources required to support the health intelligence program at three levels of funding are given in the following Table. Specific objectives, milestone dates and number of additives tested are listed in the tast statements of the Appendix.

Table

Task Description	Dollars ($1000)/Man-Years of Effort (MY)		
	Present	Minimally Adequate	Optimal
1. Review Committee	25/1	60/3	240/7
2. Effect on Emissions	—Non-health, considered elsewhere—		
3. Usage Patterns	—Non-health, considered elsewhere—		
4. Collect Exhaust Particulates	None	45/2.0	45/2
5. Toxicologic Screening System	200/4.1	610/6.5	1260/11
6. Biochemical Screening System	None	250/5	750/10
7. Trace Element Toxicology	—Biomedical Research Program Element—		
8. Controlled Human Exposures	None	100/1.7	430/6.7
9. Epidemiologic Studies	None	50/0.2	200/0.4
Total	225/5.1	1115/18.4	2925/37.1
(Intramural)	(135/5.1)	(394/18.4)	(863/37.1)
(Extramural)	(110/0)	(721/0)	(2062/0)

VI. POTENTIAL PROBLEMS

The proposed system for evaluating the health risks of specific fuels and fuel additives represents the most practical, presently feasible approach, but it is still beset by a number of perplexing problems. These belong to three broad groupings: scope of the system, relevancy of the biological test procedures and economics of testing.

The scope of the proposed system will not initially include lubricating oils, aviation fuels, diesel oil, kerosene and fuel oils or their additives even though these products are also subject to the registration procedure. Separate but analogous emissions characterization systems must be established to evaluate these products. The biological testing systems proposed by the Task Force would, however, be applicable to such products. An unanswered, but important problem, is the relative health risk resulting from trace element and organic particulate emissions from fuels and fuel additives when compared to coal-fueled stationary sources not subject to registration. Will expensive motor vehicle fuel controls be acceptable if quantitatively more important stationary sources are not controlled? Another problem with the proposed system is the use of test methods, based upon a single additive and a standard reference fuel. In practice, more than one additive might be present in any single marketed fuel blend and any single additive could be utilized with a number of different fuels. The picture is even further complicated by seasonal shifts in fuel composition, by seasonal shifts in additive use and by frequent variations in the crude oil precursors of a single market fuel. A third problem with the proposed emissions test system relates to the use of standard, carefully maintained combustion and exhaust control systems coupled to artifical irradiation chambers.

Significant variations from this system would certainly be found if studies focused upon operating vehicles. The Task Force recognized the need to quantitate these sources of variation, but held firm in its conviction that the proposed emissions and biological test systems should be developed as soon as possible and modified as needed later.

Many questions can be raised about the relevancy of the proposed biological test systems. First, there are questions regarding pollutant exposure. Should test systems not be exposed simultaneously to gaseous and particulate emissions? The Task Force felt that such interaction studies might be desirable, but were not feasible for toxicologic screening. We could not justify at this time the substantial additional investment in facilities and personnel or the increase in logistical complexity that would result. Should test systems not utilize single compound exposures instead of the complex particulate mixture or trace element exposures now planned? Again the Task Force felt that the very large number of organic compounds precluded the single compound approach in the screening process. Should potential exposures from partially combusted fuel additives and evaporative losses receive greater emphasis? The Task Force thought not in view of rapid changes in evaporative and exhaust controls and the elapsed time which will occur before testing systems can become functional.

The relevance of the toxicologic screening tests can also be questioned. Effects on cell cultures, increased susceptibility to respiratory infection, mutagenesis, carcinogenesis, impaired enzymatic function and trace element toxicity are the most significant, but not the only adverse health effects of fuels and fuel additives. Other health indicators could be added if experience indicates additional significant health risks or if additional resources become available. The proposed screening tests provide feasible indices of suspicion,

but are neither perfectly sensitive nor perfectly specific. As new knowledge develops, other screening procedures can be substituted. The lack of perfect screening test is not a legitimate excuse to delay biologic testing.

The proposed testing system is not inexpensive. Industry should pay for emissions characterization and the collection of exhaust particulates before and after irradiation for use in biological testing. If possible, it would be best for the Federal Government to sponsor the necessary biological testing utilizing an extremely small portion of the Federal tax on fuels. This dual strategy would result in the least economic dislocation to industry, which already utilizes test procedures closely akin to those now visualized for emissions characterization. Industry has little or no experience with the proposed toxicologic screening system and this system will likely further evolve during the next few years. Industry might, however, be required to support and perform additional standard toxicologic testing of additive components and surveillance of occupational groups exposed during manufacturing and distribution.

The Federal Government should support the infrequent, but necessary, tests involving human volunteers and any epidemiologic investigations. The proposed tax of .01¢ per gallon of gas would amply support the establishment and improvement of the biologic testing system. When compared to the economic importance of fuels and fuel additives, the proposed testing costs are more than reasonable. Remember, the health insurance purchased by the recommended program benefits the entire population of our nation.

APPENDIX

Task Statements Relating to Health Intelligence for Fuel and Fuel Additive Registration

Task 1. Establish Fuel and Fuel Additive Review and Recommendation Committee.

A. Task Objective Statements:

1. To identify fuels and fuel additives which require further health and impact evaluation before registration is permitted.
2. To identify relevant health and environmental impact testing systems for fuels and fuel additives.
3. To constantly re-evaluate currently registered additives.

B. Approach:

Representatives from the following EPA groups should participate in this committee:

Office of Fuel and Additive Registration
Division of Health Effects Research
Division of Chemistry and Physics
Division of Ecology
Office of Research and Monitoring
Office of Air Programs

Consider each registered fuel or additive and select some for further testing, based on predicted effects on emissions or control devices, extent of use, and postulated public health and welfare hazards.

C. Required Resources for Each Objective:

FY 73 Resource Estimate: $1000/MY

	Present	Minimally Adequate	Optimal
1. Review of fuels and additives submitted for registration	9/0.4	30/1.5	60/3.0
2. Identify testing systems	8/0.3	10/0.5	120/1.0
3. Re-evaluate registered additives	8/0.3	20/1.0	60/3.0
	25/1.0	60/3.0	240/7.0
Allocation of Resources			
In-House	$25,000	$60,000	$140,000
Contracts	0	0	$100,000

D. Milestone Events/Target Dates for each Objective:

	Target Dates at 3 Funding Levels		
	Present	Minimally Adequate	Optimal
1. Review present registered additives	1/73	10/72	10/72
2. Develop and approve testing systems:			
- existing protocols	2/73	10/72	10/72
- new protocols	-	-	1/73
3. Begin evaluating new additives	6/73	4/73	1/73

TASK 2. Identify Effects of Additives on Emission Products

A. Objective:

The Division of Chemistry and Physics has program responsibility to determine the effects of fuels and fuel additives on emission products. Specific task objectives which will result in test protocols are:

1. Determine the effect of fuel and fuel additives on exhaust particulate emissions.

2. Determine the effect of fuel and fuel additives on exhaust gas emissions.

3. Determine the effect of fuel and fuel additives on the performance of emissions control devices.

4. Determine the effect of fuel and fuel additives on atmospheric visibility.

5. Develop a predictive emissions model which allows an assessment of fuel and fuel additive effects on exhaust products by analysis of intermediary combustion products.

B. Approach:

Effects research efforts by DCP are aimed at developing protocols utilizing specified base fuels and additives, automotive engines, engine operation procedures, and gaseous and particulate collection procedures to allow the assessment of the effects of fuels and fuel additives on gaseous and particulate emissions, performance of control devices, and atmospheric visibility (secondary particulates). An additional approach is aimed toward the development of a relatively fast screening method to determine potential exhaust species and the effect of fuels and additives thereon by analysis of combustion intermediate species thus allowing rapid and economical assessment of potential toxic emission products.

The approach would involve collection and analysis of gaseous emission products. Additionally, it would involve collection and analysis for total mass emitted, trace metals, and organic fractions of both fresh and irradiated exhaust particulate. Collected particulate materials would be available for subsequent toxicological screening.

C. Resources: (Outside of Funds for Health Intelligence)

FY '73	In-house manpower	$200,000
	Equipment	225,000
	Contract program	350,000
	Total	$775,000

D. Milestone Events/Target Dates:

FY 73 A functioning emissions characterization system which will allow collection of fresh and irradiated exhaust particulates.

Task 3. Fuel and Additive Usage Data

A. Task Objective Statement:

To obtain information on the use of individual fuels and fuel additives, in order to determine magnitude of impact of harmful emissions on the environment.

B. Approach:

The direct approach, recommended by this Task Force, is to request legislation to provide the authority to obtain from fuel additive manufacturers their production of each additive and from fuel manufacturers their use of each additive in, and their production of, each designated fuel.

Lacking such legislative authority, preliminary contact has been made with the Air Quality Management Branch, OAP, to develop fuel usage by manufacturer for combination with fuel and additive registration data to provide total usage of a fuel, an additive, or a chemical compound.

C. Required Resources: (outside of funds for Health Intelligence)

		In House	Contract	Total
FY 1973	1/2 man-year	10,000	90,000	100,000

D. Milestone Events/Target Dates:

9/1/72 Contract awarded.

3/1/73 Arrangements for receipt of State data completed.

7/1/73 Usable data on fuel usage available.

TASK 4. Extracts of Emissions Resulting from Use of Selected Fuel Additives for Toxicologic Screening

A. Task Objective Statement:

Collect and provide for use in toxicologic screen test systems exhaust particulates associated with the use of selected fuel additives.

B. Approach:

Using a standard fuel engine atmosphere generation system, produce successive test atmospheres (irradiated and non-irradiated) by combustion of reference fuel with and without single selected fuel additive products (MCT, etc.), followed by extracting particulate material and forwarding for testing in a rapid toxicologic screening system (Task 5).

C. Required Resources:

	FY 73 Resource Estimate ($1000/MY)		
	Present	Minimally Adequate	Optimal
Allocation: In-house	0/0	$45/2	45/2
Contract	-	-	-

D. Milestone Events/Target Dates:

	Present	Min. Adequate	Optimal
Initial extracts for reference fuel alone and with MCT	-	7/72	7/72
Remaining extracts corresponding to 4 or 5 additional selected additives	-	9/72	9/72

(Note: On line toxicologic testing in laboratory animals of emission atmospheres from above generation system is proposed under Biomedical program element support for FY 73.)

TASK 5. Toxicologic Evaluation of the Contribution of Fuel Additives to Vehicular Exhaust

Objective:

To detect biological effects of exhaust particulates by a sequential application of a variety of biological models. The source of particulates for biological exposure is to consist of an engine dynamometer reaction chamber, designed and built, or adapted to collect particulate emissions and operated according to a standard protocol.

SUBTASK 1.

A. Task Objective Statement:

Screen particulates derived from the above source in tissue culture.

B. Approach:

Determine comparative toxicity by means of selected biological and biochemical parameters on material collected from combustion of a referenced gasoline, alone and after addition of the fuel additive, and before and after reaction in the irradiation chamber.

C. Required Resources for Each Objective:

		Resource Estimate: $1000/MY		
		Present (FY '73)	Minimally Adequate	Optimal
		$ 50/1.0	$ 100/1.0	$ 350/2.0
Allocation of Resources	- In-House	$25	$25	$ 50
	- Contract	25	75	300

D. Milestone Events/Target Dates for Each Objective:

Objective	Milestone	Target Dates at 3 Funding Levels Present	Minimally Adequate	Optimal
Screen in tissue culture	Exploratory work	10/72	10/72	10/72
	Design protocol	1/73	1/73	1/73
	Negotiate contract	3/73	3/73	3/73
	Data collection & report			
	1 additive	12/73	10/73	8/73
	5 additives	-	8/74	6/74
	15 additives	-	-	12/74

Subtask 2. Determination of the Effect of Fuel Additives, Selected on Basis of Reaction in Subtask 1 Above, Stepwise in a Number of Definitive Systems to Further Delineated the Toxicology and Validate Results in Subtask 1.

A. TASK OBJECTIVE STATEMENTS:

1. Test the effect of fuel additives on pulmonary alveolar macrophages.
2. Test the effect of additives on a in vitro mutagenic model.
3. Test the effect of fuel additives on a carcinogenesis model in newborn mice.
4. Test, etc. on enhancement of pulmonary infection.
5. Test, etc, on production of dominant lethal mutations in mice.
6. Test, etc, on production of pulmonary tumors by intratracheal instillation in hamsters.

B. APPROACH:

1. Material would be placed in surviving cultures of pulmonary alveolar macrophage and effect would be noted on selected biological and biochemical parameters.

2. Material would be studied for its property to induce mutagenesis in yeast or mold cultures.

3. Material would be injected subcutaneously in newborn mice which would be sacrificed and studied pathologically after an appropriate latency period.

4. Material would be introduced into the lungs of mice to be proceeded or followed by infectious organism. Parameters of clearance, bacterial growth and mortality would be observed.

5. Material would be injected into male mice which would then be bred, and females examined by standard procedure to detect presence of dominant lethal genes in mice.

6. Material would be injected into tracheal of hamsters lone, with inert particles and with known carcinogens. Animals would be sacrificed and examined for tumors after period of latency.

C. REQUIRED RESOURCES FOR EACH OBJECTIVE:

Resource Estimate: $1000/MY

Objectives		Present	Minimally Adequate	Optimal
1. Toxicology in P.A.M.		$20/1.0	$80/1.0	$150/2.0
Allocation of Resources	- In-House	$20	$20	$40
	- Contract		$60	$110
2. Mutagenesis in vitro		$20/0.2	$40/0.5	$100/1.0
Allocation of Resources	- In-House	$5	$10	$20
	-	$15	$30	$80

Objectives		Present	Minimally Adequate	Optimal
3. Carcinogenesis in newborn mice		$50/0.5	$120/1.0	$150/1.0
	Allocation of - In-House	$10	$30	$30
	Resources - Contract	$40	$90	$120
4. Infectivity studies		$20/1.0	$80/1.0	$160/2.0
	Allocation of - In-House	$20	$20	$40
	Resources - Contract	0	$60	$120
5. Dominant lethal in mice		$20/0.2	$120/1.0	$230/2.0
	Allocation of - In-House	$5	$20	$40
	Resources - Contract	$15	$100	$190
6. Intratracheal		$20/0.2	$70/1.0	$120/1.0
	Allocation of - In-House	$5	$20	$20
	Resources - Contract	$15	$50	$100

D. MILESTONE EVENTS/TARGET DATES FOR EACH OBJECTIVE:

Objective	Milestone	Target Dates at 3 Funding Levels Present	Minimally Adequate	Optimal
1. Toxicity in PAM	Design protocol	12/72	12/72	12/72
	Negotiate contract	3/73	3/73	3/73
	Data collection & report:			
	1 additive	12/73	12/73	12/73
	10 additives	--	12/74	6/74
	20 additives	--	--	12/74
2. Mutagensis in vitro	Design protocol	12/72	12/72	12/72
	Negotiate contract	3/73	3/73	3/73
	Data collection & report:			
	2 additives	12/73	12/73	12/73
	10 additives	--	12/74	6/74
	20 additives	--	--	12/74

Objective	Milestone	Target Dates at 3 Funding Levels: Present	Minimally Adequate	Optimal
3. Carcinogenesis in newborn mice	Feasibility studies	12/72	12/72	12/72
	Design protocol	2/73	2/73	2/73
	Negotiate contract	3/73	3/73	3/73
	Data collection & report:			
	1 additive	6/74	6/74	6/74
	10 additives	--	10/74	10/74
	20 additives	--	--	6/75
4. Infectivity studies	Design protocol	10/72	10/72	10/72
	Negotiated contract	3/73	3/73	3/73
	Data collection & report:			
	1 additive	12/73	12/73	12/73
	5 additives	--	6/74	6/74
	15 additives	--	--	12/74
5. Dominant lethal test in mice	Design protocol	12/72	12/72	12/72
	Negotiated contract	3/73	3/73	3/73
	Data collection & report:			
	1 additive	12/73	10/73	8/73
	6 additives	--	8/74	6/74
	12 additives	--	--	12/74
6. Intratracheal inoculation of hamsters	Design protocol	6/73	6/73	6/73
	Negotiate contract	12/73	12/73	12/73
	Data collection & report:			
	1 additive	12/75	12/74	12/74
	5 additives	--	12/75	6/75
	12 additives	--	--	12/75

TASK 6. Use of Selected In Vitro Enzyme Assays as Indicators of Auto Exhaust Toxicity.

A. TASK OBJECTIVE STATEMENTS:

The object of this task is to develop a series of twenty-five in vitro enzyme assays which can be used to study auto exhaust component toxicity. The enzymes chosen will constitute a spectrum that focuses on many aspects of general metabolism or serve as points of metabolic control in critical pathways. This assay protocol will be used as a back-up to the whole cell system task and will serve to evaluate selected examples prior to their study in whole animal specific models. Findings in these enzyme systems will serve as more specific indicators and quantifiers of component toxicity and thus, will provide a more comprehensive definition of component toxicity in all test systems.

B. APPROACH:

The approach will involve the development of a series of in vitro enzyme assays to serve as test systems in the specific evaluation of auto exhaust component toxicity.

These assays will serve as a further refinement of the systems outlined in Task 5 and will be applied only to samples shown to have toxicity in these preliminary toxicological assay systems. It may not be necessary in every case to use the entire spectrum of enzyme assays, but if needed they can be applied.

Components will be added in graded doses to the suitable enzyme preparation and the IC50 and IC90 (50% and 90% inhibition concentration) will be determined. If a component is shown to have significant toxicity for a specific enzyme or class of enzymes studies of its inhibitory kinetic profile will be made. In this case components will be classed as competitive or non-competitive and attempts will be made to define the molecular basis of competition.

The enzymes selected for use in this system appear below:

Enzyme	Class	Metals	Cofactors
Acetyl Choline Esterase	Hydrolase		
Pancreatic Lipase	Hydrolase	Ca	
Ribonuclease	Nucleotidyl Transferase		
Phosphorylase b	Hexosyl Transferase		PLP
Amylase	Glucoside Hydrolase	Ca	
Leucine Aminopeptidase	Aminopeptide aminoacid hydrolase	Zn	
Carboxypeptidase A	Carboxypeptide Aminoacid hydrolase	Zn	

Alcohol Dehydrogenase	Oxidoreductase	Zn	NAD
Lactic Dehydrogenase	Oxidoreductase	Zn	NAD
Glucose-6-Phos. Dehydrogenase	Dehydrogenase		NADP
Cytochrome Oxidase	Oxidase	Fe, Cu	
Xanthine Oxidase	Oxidase	Mo, Fe	
Tyrosinase	Oxidoreductase	Cu	
Carbonic anhydrase	Hydro-lyase	Zn	
Glucose Phosphate Isomerase	Isomerase		
Phosphoglucomutase	Phosphotransferase	Mg	HDP
Superoxide Dismutase	Dismutase	Zn, Cu	
Alkaline Phosphatase	Phosphohydrolase	Zn	
Hexokinase	Phosphotransferase	Mg	ATP
Creatine phosphokinase	Phosphotransferase	Mg	ATP
Phosphofructokinase	Phosphotransferase	Mg	ATP
Aspartate Aminotransferase	Amino Transferase		PLP
Alanine Aminotransferase	Amino Transferase		PLP
Phosphoribosyl Transferase	Transferase		
Dihydrofolate Dehydrogenase	Oxidoreductase		NADP

C. REQUIRED RESOURCES FOR EACH OBJECTIVE:

Resource Estimate: $1000/MY

Objectives	Present	Minimally Adequate*	Optimal*
All Enzyme Assays	0/0	250/5.0	750/10.0
Totals	0/0	250/5.0	750/10.0
Allocation of Resources - In-House		100/5.0	300/10.0
Resources - Contract		150/0	450/0

*This resource figure includes only the funds needed to set-up and refine all assay systems

**The cost per unit sample carried through the entire assay protocol is calculated to be $2000/sample.

D. MILESTONE EVENTS/TARGET DATES FOR EACH OBJECTIVE:

Milestone Events	Target Dates at 2 Resource Levels	
	Minimally Adequate	Optimal
Negotiate contract	12/72	12/72
Develop Assays In-House	3/73	3/73

TASK 7. Trace Element Inhalation Toxicology of Combustion Products from Fuel Additives

A. Task Objective Statements:

1. Modify existing aerosol generation system for the combustion of fuel additives.

2. Characterize and define exposure chamber atmospheres.

3. Quantitate in laboratory animals the impact of trace elements (initially Mn) present in the additives upon normal body burdens. Determine the uptake, excretion and tissue levels of the trace metals.

4. Evaluate specific physiologic functions as indicated based on anticipated biological effects of the combustion products. For example, behavioral changes and neurological should be evaluated following exposure to additives containing Mn.

B. Approach:

1. A simple combustion system such as a jet flame will be incorporated into our existing inhalation exposure system.

2. The atmospheres will be characterized as to the gaseous components such as CO and hydrocarbons, particulate mass and size; and quantitation of the trace metal of concern.

3. Following exposure of the animals, trace metal determinations will be made on a selected group of tissues along with determination of the amounts in the diet, feces and urine. The tissue values will be compared with those obtained from control animals exposed to ambient levels.

4. As indicated by the compound under study, certain other physiological parameters will be evaluated so as to determine the effects of increased levels of exposure.

C. Required Resources for each Objective:

Funded under the Biomedical Research Program Element.

D. Milestone Events/Target Dates of Each Objective:

1. Modification of Equipment 9/72
2. Testing and Atmospheric Characterization of the System 11/72
3. Exposures and data collection 12/72-3/73
4. Analysis and report 4/73

TASK 8. Controlled Human Exposures to Components of Auto Exhausts

A. TASK OBJECTIVE STATEMENTS:

1. Test the eye irritation potential of auto exhaust components resulting from use of new fuels or additives registered with the Office of Fuels and Fuel Additive Registrations.
2. Quantitate in humans uptake, excretion and tissue levels of trace elements present in fuels and additives and emitted from advanced control systems.
3. Test in humans adverse effects of controlled exposure to auto exhaust components upon resistance to infectious challenge, after demonstration of such effects in animal model systems.

B. APPROACH:

1. Eye irritation studies: graded doses of exhaust components will be tested against tear lysozyme levels and subjective sensation of eye irritation at exposures bracketing ambient levels resulting from projected use of new fuels or additives.
2. Trace element studies: careful measurement of trace element in food will be coupled to controlled inhalation after vaporization of the element in jet flame. Amounts taken in, excreted in feces and urine, and concentrations in blood and hair will be quantified. Potential for increased body burdens from ambient levels produced by exhaust emissions will be defined. Effects on activity of selected enzymes in blood will be determined.
3. Infectivity studies: respiratory challenge with common benign respiratory viruses and bacteria will follow carefully dosimetered exposure to auto exhaust components found to adversely affect animal resistance to infectious challenge.

C. REQUIRED RESOURCES FOR EACH OBJECTIVE:

	FY 73 Resource Estimate: $1000/MY		
Objectives	Present	Minimally Adequate	Optimal
1. Eye irritation	0/0	$20/0.4	$50/1.0
2. Trace elements	0/0	80/1.3	250/4.0
3. Infectivity	0/0	0/0	100/1.7
Totals	0/0	$100/1.7	$400/6.7
Allocation of Resources - In-House		$40	$100
Allocation of Resources - Contract		$60	$300

D. MILESTONE EVENTS/TARGET DATES FOR EACH OBJECTIVE:

1. Eye irritation

Milestone Events	Target Dates at 2 Resource Levels: Minimally Adequate	Optimal
Negotiate contract	12/72	12/72
Data collection - one additive	3/73	3/73
-second additive	--	4/73
-third additive	--	5/73
Analysis and report	5/73	5 to 7/73

2. Trace element studies

Milestone Events	Target Dates at 2 Resource Levels: Minimally Adequate	Optimal
Negotiate contract	10/72	10/72
Data collection - one element	2/73	2/73
-second element	4/73	2/73
-third element	--	4/73
-fourth element	--	4/73
-fifth element	--	6/73
-sixth element	--	6/73
Analysis and report	6/73	6 to 9/73

3. Infectivity studies

Milestone Events	Target Dates at 2 Resource Levels: Minimally Adequate	Optimal
Negotiate contract	--	1/73
Data collection - one element	--	3/73
-second element	--	5/73
Analysis and report	--	7/73

TASK 9. Epidemiologic Studies Necessary to Predict the Health Impact of Fuels and Fuel Additives

A. TASK OBJECTIVE STATEMENTS:

1. To elucidate changes in human pollutant burdens which might result from use of specific fuels and fuel additives.
2. To link changes in human pollutant burdens to alterations in physiology and increased risk of acute or chronic diseases.

B. APPROACH:

1. Changes in human pollutant burdens might be predicted by special study of populations already representing an exposure gradient for the pollutants in question. This approach would be most appropriate for trace element exposures, the most important of which are manganese, nickel and chromium. Tissues and body fluids would be collected from volunteers and at necropsy. The levels of selected trace metals would then be assayed by appropriate techniques. Information from these studies would be interfaced.
2. Human pollutant burden levels in selected cases could be linked to changes in metalloenzyme activity, to appropriate indices of erythrocyte metabolism, to changes in neurophysiology, alterations in performance testing, and to cytogenetic abnormalities in circulating lymphocytes and respiratory epithelium.

C. REQUIRED RESOURCES FOR EACH OBJECTIVE:

FY73 Resource Estimate: $1000/MY

Objectives	Present	Minimally Adequate	Optimal
1. Human pollutant burden	0/0	$50/0.2	$100/0.2
2. Pathophysiologic studies	0/0	0/0	100/0.2
Total	0/0	$50/0.2	$200/0.4
Allocation of Resources - In-House		-4-	-8-
Resources - Contract		$46	$192

D. MILESTONE EVENTS/TARGET DATES FOR EACH OBJECTIVE:

1. Human pollutant burden

Milestone Events	Target Dates at 2 Resource Levels: Minimally Adequate	Optimal
Negotiate contract	12/72	12/72
Data collection: one trace element	6/73	6/73
two trace elements	--	6/73
Analysis and report	12/73	12/73

2. Pathophysiologic studies

Milestone Events	Target Dates at 2 Resource Levels: Minimally Adequate	Optimal
Negotiate contract	--	12/72
Data collection: one trace element	--	12/73
two trace elements	--	--
Analysis and report	--	3/74